Research Article

Chemical Constituents from *Macaranga peltata* Roots and Screening of their Cytotoxic Activity

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ABSTRACT

Chemical constituents from *Macaranga peltata* roots and screening of their cytotoxic activity

Phytochemical investigation of $CHCl_3$ extract of the roots of *Macaranga peltata* led to the isolation of siaresinolic acid **(1)** along with four other known compounds lupeol, sitosterol, stigmasterol and oleanolic acid (**2**-**5**). The occurrence of siaresinolic acid is reported for the first time from the root of *Macaranga peltata*. The compounds were identified and characterized on the basis of various spectral techniques including 1D and 2D NMR. In addition, all the isolates and different solvent extract were tested for their cytotoxicity against the cervical cancer (HeLa), breast cancer (MCF-7), and prostate cancer (PC-3) cell lines using the MTT assay.

Keywords: *Macaranga peltata*; Euphorbiaceae; Siaresinolic acid; Cytotoxicity.

INTRODUCTION

Macaranga peltata Muell syn. *Macaranga roxburghii,* a resinous tree belonging to the family Euphorbiaceae, is widely distributed in South East Asia especially Thailand, Sri Lanka and India. It is commonly known as Chandada or Chand Kal in Hindi and Boddi in Telugu. Traditionally, the bioactivity of many Macaranga species has been harnessed in folk medicine in the tropical regions. The gum powder of the plant is reported to be useful in the treatment of veneral diseases[1]. Previous phytochemical studies of the heartwood and stem bark of this medicinally important plant has resulted in isolation of bergenin along with its derivatives, cyclopeltenyl acetate, ellagic acid and other triterpenes, flavonoids, chalcones etc[1-3]. As part of our continuing efforts in the isolation of structurally interesting and of biologically active compounds from the Indian medicinal plants[4-7], we report isolation, structure elucidation of the compounds isolated from $CHCl_3$ extract of *M. peltata* roots along with their biological activity against human cancer cell lines. To the best of our knowledge it is the first report on isolation of chemical constituents of the *M. peltata* roots. The compounds were identified and characterized on the basis of various spectral techniques including 1D and 2D NMR.

EXPERIMENTAL

General Procedures

Melting points were recorded on a Fisher scientific melting point apparatus and were uncorrected. Mass spectra were recorded on a Agilent LC/MSD trap SL 1100 series with a 70 ev (ESI probe) and IR spectra were recorded on a Thermo Nicolet Nexus 670 FTIR spectrometer. ^{1}H-NMR, ^{13}C-NMR, DEPT and 2D-NMR (HSQC, HMBC and COSY) experiments were recorded on a Bruker 300 MHz spectrometer using TMS as an internal standard. All the solvents used were of analytical grade. Column chromatography was performed on silica gel (60-120mesh, Acm's make, Mumbai, India). Thin layer chromatography (TLC) was performed on Merck silica gel $60F_{254}$ plates (E. Merck, Darmstadt, Germany). Visualization was performed by spraying the TLC plates with 5% H_2SO_4 solution followed by heating.

Plant Material

The roots of *M. peltata* were collected from Tirumala forest, Tirupathi, Andhra Pradesh,

India and identification was made by Prof. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupathi. A voucher specimen of the plant has been deposited in the herbarium, Department of Botany with an accession number, 575.

Extraction and isolation

The dried roots (4kg) were grinded and extracted three times with $CHCl_3$ in a soxhlet apparatus for approx. 72 hours. The resulting extract was then concentrated under vacuum to obtain a residue. The residue was dissolved in hot methanol and left overnight at room temperature. On cooling insoluble waxes which separated out, were removed by filtration and methanol soluble part was concentrated to give a residue (7.1gm). This residue was subjected to column chromatography over silica gel (60-120 mesh) using an eluent system of increasing polarity of n-hexane/ethyl acetate to yield six major fractions (F1-F6). Fraction F2 was subjected to repeated silica gel (60-120 mesh) column chromatography by eluting with ethyl acetate and hexane (1.2:8.8) to yield compound **2** (lupeol, 65mg). Fraction F3 was subjected to repeated column chromatography using ethyl acetate and hexane (1.5:8.5) as elutents to yield compound **3** (sitosterol, 158mg). Fraction F4 was crystallized with methanol to yield compound **4** (stigmasterol, 70mg). Fraction F5 was subjected to repeated column chromatography by eluting with ethyl acetate and hexane (2.8:7.2) to yield compound **5** (oleanolic acid, 28mg); with ethyl acetate and hexane (3.1:6.9) to yield compound **1** (siaresinolic acid, 45mg).

The different extracts were prepared by cold percolation technique using solvents of varying polarity and were designated as HEC (hexane), CEC ($CHCl_3$), MEC (MeOH) and WEC (H_2O). In addition for hexane and $CHCl_3$ solvents, hot extraction technique involving use of soxhlet was also applied to obtain HEH (hexane), CEH ($CHCl_3$) extracts respectively.

Siaresinolic acid (1)

white amorphous powder; mp 188-190^{0}C; ESIMS: *m/z* 471 $(M-H)^-$; IR (KBr, v, cm^{-1}): 3598 (OH), 1685 (COOH); for the ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), (See Table-1).

Biological assay

Cytotoxicity was analyzed by MTT assay[12] using Doxorubicin as control. (Table 2).

RESULTS AND DISCUSSION

The methanol fraction of the $CHCl_3$ extract of *M. peltata* roots was column chromatographed on silica gel to afford the compounds **(1-5)**. The compounds were identified as lupeol (**2**)[8], sitosterol (**3**)[9]**,** stigmasterol (**4**)[9], oleanolic acid (**5**)[10] and siaresnolic acid (**1**)[11], from ^{1}H and ^{13}C NMR data which were compared with those reported in literature.

Compound **1** was obtained as a white amorphous powder. A molecular ion peak at m/z 471$[M^+-H]$ in the ESIMS of **1** in conjunction with its ^{13}C NMR spectrum established the molecular formula of **1** as $C_{30}H_{48}O_4$. IR spectrum displayed sharp bands at 3598 cm^{-1} suggesting the presence of hydroxyl group and at 1685 cm^{-1} for carboxyl group. Its 300 MHz ^{1}H NMR spectrum showed a triplet at δ 5.44 integrating for one proton due to the double bond and oxygenated methine protons at δ 3.22 and 3.33. Additionally, seven singlet methyl signals at δ 0.98, 0.79, 0.90, 0.70, 1.25, 0.98 and 0.96 were also observed. The above data suggested that compound could be an oleanane type triterpenoid. The ^{13}C NMR spectrum of the compound in $CDCl_3$ indicated the presence of thirty carbon signals (Table-1) including two oxygenated methine at δ 79.0 and δ 81.50. It further displayed signals at δ 142.5 corresponding to a quaternary carbon of a double bond and at δ 183.4 due to carbonyl carbon of carboxylic group. The combined ^{1}H and ^{13}C NMR data, together with DEPT, HSQC and HMBC experiments suggested the presence of seven methyl groups (δ 28.0, 15.5, 15.2, 17.2, 25.1 28.0, 24.4), nine methylenes (δ 38.2, 27.4, 18.5, 32.5, 23.8, 29.7, 32.5, 32.5, 28.0), six methines (δ 79.0, 55.2, 47.8, 125.4, 43.5, 81.5) and eight quaternary (δ 39.6, 38.8, 37.2, 142.5, 41.2, 45.2, 34.6, 183.4) carbons in the molecule. Thus, the structure of **1,** was found to be consistent to the reported literature values of known compound siaresinolic acid[11]. This was further supported by the key COSY and HMBC correlation as shown in figure 1. The isolation and identification of siaresnolic acid **(1)** from the root of *Macaranga peltata* is being reported from this genus for the first time.

All the isolated compounds **(1-5)** and solvent extracts prepared were tested for *in-vitro* cytotoxicity against cancer cell lines and IC_{50} values were calculated in micromoles (µM)[12]. The cell lines used for this study are the cervical cancer (HeLa), prostate cancer (PC-3) and breast cancer (MCF-7). The results (Table 2) showed that the activity observed at extract level was better than at individual compound level. Of all the tested compounds only

siaresinolic acid **(1)** showed significant activity against all the tested cell lines. Amongst the extracts, except for hexane percolated (HEC) extract all other extracts showed moderate to significant activity against the tested cell lines.

CONCLUSION

Thus chromatographic separation of chloroform extracts of *M. peltata* roots resulted in isolation and characterization of five known compounds. As significant cytotoxic activity was observed at extract level but not at individual compound level, further studies are needed to be carried out on the chemical constituents of this medicinally important plant species to identify the molecules responsible for its cytotoxic activity.

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Table 1: The ^{1}H NMR and ^{13}C NMR data of 1 ($CDCl_3$, δ, ppm, J/Hz)

Position	δ_H	δc
1	1.58 (m)	38.2
2	2.08 (m)	27.4
3	3.22(dd, *J*=10.68, 5.61)	79.0
4	-	39.6
5	0.76 (m)	55.2
6	1.56-1.42 (m)	18.5
7	1.42 (m)	32.5
8	-	38.8
9	1.65 (m)	47.8
10	-	37.2
11	1.94 (m)	23.8
12	5.44 (t, *J* = 6.98)	125.4
13	-	142.5
14	-	41.2
15	2.11(m)	29.7
16	2.28 (m)	32.5
17	-	45.2
18	3.08 (brs)	43.5
19	3.33(d, *J* = 3.74)	81.5
20	-	34.6
21	2.11(m)	27.1
22	2.20 (m)	32.5
23	0.98 (s)	28.0
24	0.79 (s)	15.5
25	0.90 (s)	15.2
26	0.70 (s)	17.2
27	1.25 (s)	25.1
28	-	183.4
29	0.98 (s)	28.0
30	0.96 (s)	24.4

Assignments were based on 2D NMR including COSY, HSQC and HMBC. Well- resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses. For overlapped signals, only chemical shift values are given.

Table 2: Cytotoxic effects (IC_{50}, µg/mL) of the different solvent extract and all the compound isolated from *M.peltata*

Extract/Compound	Cell Lines		
	HeLa	MCF-7	PC3
HEC	60±6.3	34±4.5	41±12.1
CEC	32±2.1	15±2.7	11±0.65
MEC	17±1.7	24±2.9	12±0.55
WEC	17±2.4	28±2.9	23±1.2
HEH	18.9±2.3	10±2	21±1.4
CEH	15±2.1	27±5.2	18±1.7
Siaresinolic acid	25±3.6	21±2.1	19±4.2
Lupeol	58±4.9	79±8.9	41±2.7
sitosterol	57±3.8	58±9.1	90±8.9
Stigmasterol	37±0.98	100±15	31±3.2
Oleanolic acid	97±11.1	74±1.9	54±5.5
Doxorubicin	3±0.45	2.71±0.87	4.2±0.51

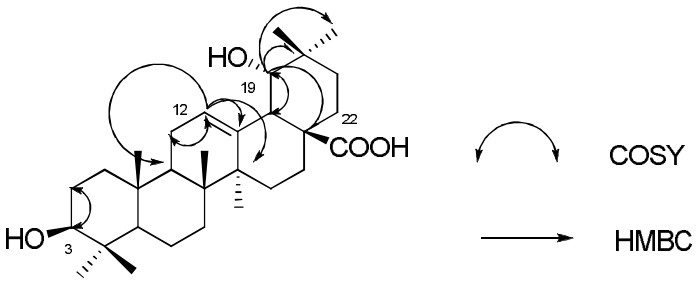

Figure 1: Key COSY and HMBC correlations of Siaresinolic acid **(1)**

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